Histopathological evaluation of the effects of live Infectious bursal disease vaccine originated from WF2512 strain on bursa Fabricius in the broilers

ABSTRACT

Infectious bursal disease (IBD) is a viral disease that causes significant economic losses in young chickens, characterized by lymphoid depletion and inflammation in the bursa Fabricius (BF). The incidence of the disease shows an increasing trend all over the world. Active and passive immunization is very important as well as strict hygiene measures in combating outbreaks. However, the fact that live-attenuated vaccines (mild, intermediate, hot) used for this purpose cause immunosuppression because of bursal damage is seen as an important limitation. In this study, it was aimed to histopathologically investigate the effects of commercial IBD vaccines originating from WF2512 (intermediate plus/hot, orally with drinking water) on BF under routine broiler rearing conditions. For this, BFs of 55 Ross 308 hybrid breed chickens (50 test, 5 controls) from five different broiler farms were used. In addition to standard vaccines, the IBD vaccine was given on day 15, and five samples from each farm were obtained 10 days later (25th day). After the first sampling, the second BF sampling was performed at the age of 38 days. Histopathological bursal lesion score was applied to evaluate the effectiveness of the vaccine. Accordingly, it was determined that the bursal lesion score, which increased slightly to moderately in the first samples, decreased in the second samples (27-61%). This was accepted as an indication that the bursal damage, which increased with IBD vaccine administration, diminishes over time and that histological regeneration was increased.

Keywords: Bursa Fabricius, histopathology, immunosuppression, infectious bursal disease

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral disease that causes severe inflammation in the bursa Fabricius, progresses with immunosuppression, and causes significant economic losses in the poultry industry, especially in young chickens (Berg, 2000). Gumboro disease, also known as avian nephrosis due to kidney damage, was named after the Gumboro region where the first outbreaks occurred (Cosgrove, 1962). However, due to morphological and histopathological changes in bursa Fabricius (BF) it was later dubbed IBD (Hitchner, 1970). In the 3- to 6-week period when the development of bursa Fabricius is the fastest, the clinical form develops in chickens infected with the virulent Infectious bursal disease virus (IBDV) and the disease progresses severely. The subclinical form, in which almost no clinical signs are visible, develops at the age of less than 3 weeks. Immunosuppression develops in both the acute/clinical and subclinical forms, preventing the development of an adequate immune response to subsequent vaccinations and increasing susceptibility to secondary infections (Mazariegos et al., 1990; Müller et al., 2012).

How to cite this article

Ateş, MB., Çelik, Z., Salık, R., Dağar, O., Bulut, A., Tural, A., Özdemir Ö. (2022). Histopathological evaluation of the effects of live Infectious bursal disease vaccine originated from WF2512 strain on bursa Fabricius in the broilers. *Journal of Advances in VetBio Science and Techniques, 7*(2), 250-258. https://doi.org/10.31797/vetbio.1069954

Research Article

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Article info
Submission: 08-02-2022
Accepted: 06-07-2022
Online First: 25-08-2022
Publication: 31-08-2022

e-ISSN: 2548-1150
doi prefix: 10.31797/vetbio
- http://dergipark.org.tr/vetbio

In addition to hygiene measures, active and passive immunization play a critical role in the fight against IBD, which is one of the most important viral diseases causing economic losses in commercial chicken breeding around the world, and whose incidence is increasing and remains complex (Müller et al., 2012).

The disease's causative agent (IBDV) is an RNA virus with two strands (A and B) that belongs to the Avibirnavirus genus of the Birnaviridae family (Hon et al., 2008). IBDV is divided into two serotypes (Virulent serotype 1 and apathogenic serotype 2). Although virus neutralization tests and electrophoretically distinguish these two serotypes, they are indistinguishable in fluorescent antibody, agar gel precipitation, and Enzyme-Linked Immunosorbent Assay tests (Sapats & Ignjatovic, 2000). Five different viral polypeptides (VP) are found in the virus genome. VP2-5 is used to encode segment A, and VP1 is used to encode segment B. VP2, which contains at least two epitopes to neutralize antibodies that protect the susceptible host from IBDV, is the most preferred protein for protective immunization in poultry (Eterradossi et al., 1997). Furthermore, VP2 is involved in cell tropism, tissue culture adaptation, and IBDV pathogenicity (Brandt et al., 2001).

Primary viremia occurs via the portal circulation after faecal-oral and inhaled agents replicate primarily in intestinal-associated macrophages and lymphoid cells (Dey et al., 2019). Secondary viremia occurs after viruses reaching the bursa Fabricius replicate in B lymphocytes in the follicles. Thus, viruses that have the ability to spread to other tissues and organs cause specific clinical signs and symptoms, as well as death (Dey et al., 2019). BF is an epithelial and lymphoid organ in the poultry immune system where lymphocyte stem cells mature into mature, immunocompetent B lymphocytes. The virus primarily prefers BF, where the majority of B cells in young chickens are in the active division stage (Dey et al., 2019). Following BF infection, heterophile granulocyte and inflammatory cell infiltrates are observed, as well as lymphoid depletion characterized by degeneration and necrosis, particularly in B cells expressing immunoglobulin M (Eterradossi & Saif, 2020). Depletion of B cells and atrophy of BF in surviving birds results in immunosuppression with inadequate antibody response to other viral diseases or vaccinations (Dey et al., 2019).

In the fight against IBD, strict hygiene measures and live or inactive vaccination methods are widely used. However, because IBDV can be transmitted for up to 122 days in feed and 52 days in water, combating the disease becomes extremely difficult (Müller et al., 2012). Inactivated vaccines are widely used in breeder flocks to control IBD in many countries. Maternal antibody transfer helps protect the offspring until the adaptive immune response is fully functional in hatching chicks (Davison et al., 2008). The half-life of maternal antibodies in broiler lines is generally thought to be about 3 days (Müller et al., 2012).Therefore, it is of great importance to immunize chicks with live-attenuated vaccines to prevent IBD. Live-attenuated vaccines are referred to as mild, intermediate or intermediate plus (hot) vaccines based on their ability to cause varying degrees of histopathological lesions and are preferably administered via drinking water to induce strong cellular and humoral immunity (Dey et al., 2019). It has been reported that while mild vaccines do not cause significant bursal damage in chicks, intermediate or intermediate plus vaccines may cause severe bursal lesions (Dey et al., 2019). However, it is known that mild vaccines have a lower level of protection against maternal antibodies or the very virulent form of IBDV compared to other vaccines (Dey et al., 2019; Müller et al., 2012). This dilemma is common in the poultry industry. As a matter of fact, the damage that may occur in bursa Fabricius after

IBD vaccination may result in serious economic losses by increasing the susceptibility to secondary diseases after immunosuppression, as well as causing insufficiencies in immunization that will occur after other vaccinations.

Poultry farming is an industry that is developing rapidly all over the world and is economically significant. Especially the stress of reaching slaughter weight in a short time after hatching can cause broiler chickens to become susceptible to many diseases. IBD, which was first discovered nearly 60 years ago, is still considered one of the most serious threats to the poultry industry. The fact that live-attenuated vaccines, which are an important tool in the fight against this disease, can cause bursal damage and atrophy, is seen as a major drawback. In addition, although there are experimental or controlled field studies, studies conducted directly in the field conditions where other vaccine programs and routine breeding protocols are applied are very limited. In these circumstances, the use/selection of a vaccine that causes no or minimal damage to the bursa Fabricius is one of the challenges in the poultry industry. In this study, it was aimed to histopathologically investigate the effects of commercial vaccines (intermediate plus) originating from WF2512 on bursa Fabricius under routine broiler rearing conditions and to guide industry stakeholders in vaccine selection with the obtained data.

MATERIAL and METHOD

Animals, Feeding and Hosting

In the study used BF samples from 55 Ross 308 hybrid broilers (50 test, 5 controls) from five different farms. 16 hours of light and 8 hours of darkness were applied to the broiler farms. Water was provided via a nipple system on all farms, and feed was provided ad libitum via automatic feeders. Broiler rations were formulated to suit the National Research Council's basic requirements, which included no antibiotics, anticoccidials, or other additives (Council, 1994) (Table 1).

Table 1. Diet composition according to the growth period

Analytical components (g/kg)	Starter diet (1 to 10 d)	Grower diet (11 to 24 d)	Finisher Diet (25d to 42d)
Crude Protein	230	210	190
Crude Cellulose	37	38	38
Crude Oil	63	64	64
Crude Ash	67	68	68
Calcium	13	11	9
Phosporus	6,5	5,5	4,5
Methionine	5	4	4
Lysine	14	12	11
Energy (kcal)	3025	3150	3200

Study Design

All broilers used in the study were given inactivated Newcastle (NC) vaccine subcutaneously (SC), live Newcastle (La sota, spray), and live infectious bronchitis (IB, H120, spray) vaccines at the age of one day. NC (La sota, spray) and IB (H120, spray) vaccines were given again at 14 days. Then, at the age of 15 days, commercial IBD vaccine (intermediate plus) obtained from the WF2512 strain was mixed with drinking water and given orally to all broilers except the control group. Ten days after IBD vaccination, BF samples were taken from 5 broilers on each farm and control group (25th day, 25 tests, 5 controls). Finally, on the 38th day, a second BF sampling (25 tests) from the same farms was conducted. A summary of the trial design is given in Figure 1.

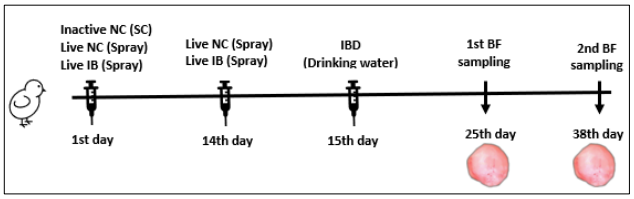

Figure 1. Study Design

Pathological Method

BF samples were fixed in 10% formol solution for 24 hours. Trimming of the hardened tissues was performed. Samples taken into tissue follower cassettes were washed in running tap water for 24 hours. An automatic tissue

processing device (Leica TP1020) was used for routine tissue processing of all tissues. After the tissues were embedded in paraffin, 5 µm thick sections were taken with a microtome (Leica RM 2125RT). The resulting slides were stained with haematoxylin-eosin (HE) (Luna, 1968). Microscopic examination was performed with light microscope and photos were taken from those deemed necessary. The method previously reported by Shaw and Davison (2000) was modified to determine the bursal lesion score. First, lymphoid depletion and accompanying inflammation findings in 10 randomly selected follicles in each BF were evaluated and scoring was done (Follicular lesion score) (Shaw & Davison, 2000). In addition, a lesion spread score (1: mild; 2: moderate; 3: severe) was determined, reflecting the overall microscopic examination of BF, and showing the frequency/spread of lesioned follicles. Finally, the numerical values obtained by multiplying these two scores (minimum:1; maximum:21) were accepted as the final score of each case. The histopathological findings based on this scoring are given in Table 2.

Table 2. Histopathological findings used in the bursal lesion score.

Follicular lesion score	Score	Lesion spread score	Score
No lesions	**1**	Mild	**1**
Interstitial mononuclear cell infiltration, edema, lymphoid depletion (<10%)	**2**	Moderate	**2**
Interstitial mononuclear cell infiltration, edema, lymphoid depletion (11-25%)	**3**	Severe	**3**
Interstitial mononuclear cell infiltration, edema, lymphoid depletion (26-40%), intraepithelial cysts	**4**		
Interstitial mononuclear cell infiltration, edema, lymphoid depletion (41-55%), intraepithelial-intrafollicular cysts, hemorrhage, mild necrosis, and fibrosis	**5**		
Interstitial mononuclear cell infiltration, edema, lymphoid depletion (56-75%), intraepithelial-intrafollicular cysts, hemorrhage, moderate necrosis, and fibrosis	**6**		
Interstitial mononuclear cell infiltration, edema, lymphoid depletion (> 75%), intraepithelial- intrafollicular cysts, hemorrhage, severe necrosis, and fibrosis.	**7**		
Total score= (Follicular lesion score) x (Lesion spread score)			

Statistical Analysis

The Kolmogorov-Smirnov test was used to analyze the histopathological scores for normal distribution. The homogeneity of variances was controlled using Levene's test. Histopathological scores were evaluated by the post-hoc Duncan test after one-way ANOVA (SPSS® Inc. version 26.0 for Windows, Chicago, IL, USA). Statistical significance was defined as a value of $p<0.05$.

RESULTS

One replicate (No:5) of Farm 1 and Farm 5 was excluded due to tissue processing and staining errors. In the histopathological examinations of BFs, varying degrees of tissue damage were detected in all vaccinated farms. Mild to severe atrophy and lymphoid depletion were determined in BF follicles. It was determined that reticular cells in the follicular became prominent. In addition to vacuoles and cystic spaces within the follicles, necrosis of varying severity was sometimes observed. In some cases, regenerative follicles were also observed. In the interfollicular region, mononuclear cell

infiltrations were seen, as well as an increase in fibrous connective tissue on occasion. There were cyst formations in the intraepithelial region, and edema in the subepithelial and interfollicular regions (Fig. 2A). Table 3 shows bursal lesion scores based on histopathological examination results of BFs examined across all farms. As a result, it was determined that the severity of lesions in Farm 1 and 5 samples taken on the 25th day decreased on the 38th day, but this was not statistically significant ($p>0.05$). In Farm-2, Farm-3, and Farm-4, it was revealed that the second samples showed a significant improvement (decrease) in bursal lesion score ($p<0.05$). The bursal lesion score in the second samples (38th day) was lower (27-61%) than in the first samples (25th day) in all farms, indicating that the bursal lesion score decreased over time (Fig. 2B-C).

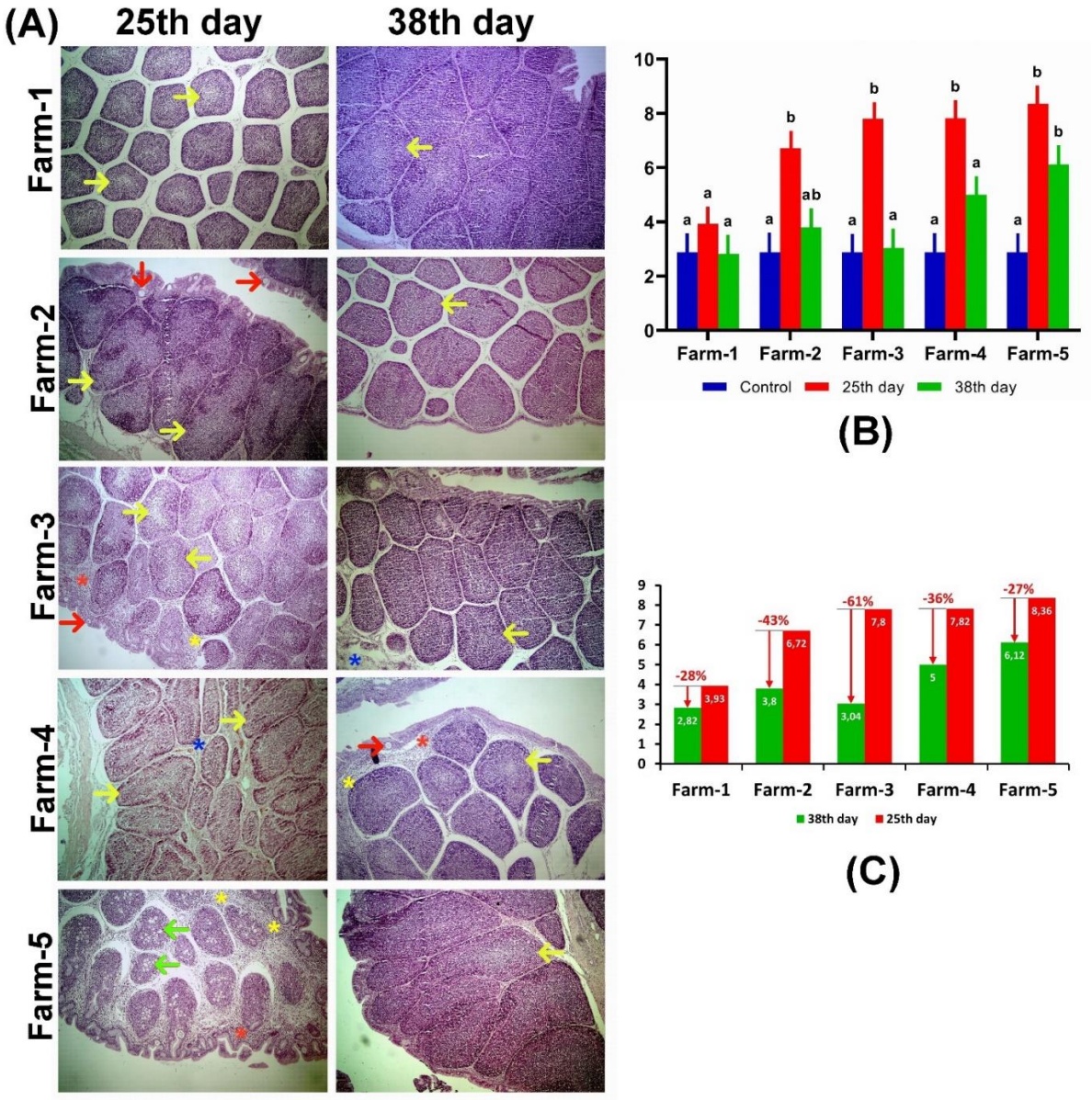

Figure 2. The effect of intermediate plus live IBD Vaccine on bursa Fabricius. (A) Representative photomicrographs of comparison of samples taken on days 25th and 38th days, bursa Fabricius, HE, 10X. Yellow arrows: Lymphoid depletion in follicle; Red arrows: Intraepithelial cysts; Green arrows: Vacuoles and cystic spaces within the follicles; Red asterisks: Inflammatory cell infiltration; Yellow asterisks: Increase in fibrous connective tissue, Blue asterisks: Edema in the subepithelial and interfollicular regions (B) Graphical representation of statistical results Changes in bursal lesion score in the 25th and 38th days. a,b The difference between the different superscripts compared to the Control group is significant ($p <0.05$, one-way ANOVA post hoc Duncan test). (C) Graphical representation of the changes in Bursal lesion scores at 25th and 38th days.

Table 3. Histopathological scoring and results

Replicate No	Control n:5	Farm-1		Farm-2		Farm-3		Farm-4		Farm-5	
		25th day n:4	38th day n:5	25th day n:5	38th day n:5	25th day n:5	38th day n:5	25th day n:5	38th day n:5	25th day n:5	38th day n:4
1	3,2	2,3	2,5	2,9	2,7	3,6	3,5	9,4	8,2	9,6	8,6
2	2,9	2,6	3,1	9,8	3,1	8,4	3,2	9	3,1	8,2	9,4
3	3	7,6	2,5	3,1	7	8,6	2,4	8,6	7	9	3,5
4	2,6	3,2	3	8,6	3,4	8,8	3	3,5	3,6	7	3
5	2,7		3	9,2	2,8	9,6	3,1	8,6	3,1	8	
Mean ±SEM	2,88 ±0,11[a]	3,93 ±1,23[a]	2,82 ±0,13[a]	6,72 ±1,53[b]	3,80± 0,80[ab]	7,80 ±1,07[b]	3,04 ±0,18[a]	7,82 ±1,09[b]	5,00 ±1,08[a]	8,36 ±0,44[b]	6,12 ±1,67[b]

[a,b]The difference between the different superscripts compared to the Control group is significant ($p < 0.05$, one-way ANOVA *post hoc* Duncan test).

DISCUSSION

Infectious bursal disease, also known as Gumboro disease, is a highly contagious viral infection that reduces the immunity of young chickens and can lead to their death at 3 - 6 weeks of age (Berg, 2000). Control of the disease is tried to be ensured with strict hygiene measures and vaccination protocols. However, epidemics that occur from time to time can cause very important economic losses in the poultry industry (Dey et al., 2019). On the other hand, the fact that the vaccines, which play a key role in this struggle, can cause immunosuppression, may lead to a decrease in the effectiveness of other vaccinations and indirect losses due to increased sensitivity to other diseases (Müller et al., 2012). This can be even more devastating in broilers reared under high yield pressure in a very short time. One of the desirable features of the vaccine to be used is that the degenerative effects on the bursa Fabricius are minimal and that regeneration takes place over time. For this reason, in this study, we focused on the histopathological investigation of the effects of commercial vaccines (intermediate plus) originating from WF2512 on bursa Fabricius in enterprises where traditional care, feeding and vaccination programs are carried out.

The histopathological bursal lesion score is one of the tests used to assess the vaccine's efficacy (Commission, 2002). However, the bursal lesion scoring scale has some drawbacks, including being too short, subjective, and difficult to optimize sampling time (Butter et al., 2003). With the development of previously used scoring methods, the bursal lesion scoring used in this study was modified. The fact that the parameters to be evaluated in the previously used methods were not very clear was considered as an important shortcoming. The current study was based on scoring the severity of different degenerative findings in randomly selected follicles during the examination of BFs. On the other hand, it was revealed that the numerical value obtained as a result of giving a general score according to the extent of these degenerative follicles and multiplying the two data as a result allows a more quantitative measurement. In fact, while degenerative changes are prevalent in some of the follicles examined in BFs, normal or even regenerative changes can be seen in others. The most important point is to determine the severity of the lesion within the degenerative follicles, as well as the extent to which these degenerative follicles have diffused.

The histopathological findings were found to be similar to those seen in prior experimental trials (Hair-Bejo et al., 2004; Henry et al., 1980; Thornton & Pattison, 1975). The degenerative alterations are caused by the vaccine's mechanism of action. Vaccine viruses almost mimic the disease and replicate in B cells, which are in the active division stage.

Meanwhile, it causes necrosis and/or apoptosis in B cells, and heterophile granulocyte and inflammatory cell infiltrations in the BF (Dey et al., 2019). Thus, an immunosuppression develops, characterized by bursal damage because of inflammation and a decrease in immunocompetent B cells, particularly affecting the humoral immune system. Some intermediate and the majority of hot vaccines, have been shown to cause severe bursal lesions, similar to those seen in IBD outbreaks (Hair-Bejo et al., 2004). However, mild and even some intermediate vaccines are known to be less effective than other vaccines at breaking a certain level of maternal antibody and protecting against the very virulent form of IBDV (vv-IBDV) (Dey et al., 2019; Müller et al., 2012). Maternal antibodies help protect offspring until the adaptive immune response is fully effective. The necessity to protect chicks in the first weeks after hatching and the high infection pressure make vaccination inevitable despite strict hygienic measures (Müller et al., 2012). Considering that immunity obtained with highly attenuated vaccine strains (mild and intermediate vaccines) cannot control outbreaks caused by vv-IBDV strains, the use of low attenuated vaccine strains (intermediate plus/hot vaccines) may become necessary in high-risk situations (Müller et al., 2012) . All these reasons complicate the selection of vaccines that are formulated to provide both minimal bursal tissue damage and optimal protection.

In a study conducted in broiler chickens, the bursa lesion scoring that occurred as a result of live intermediate vaccination on the 14th day changed from mild to moderate on the 28th and 35th days, but returned to mild on the 42nd day (Hair-Bejo et al., 2004). Similarly, both some literatures and vaccine manufacturers report that intermediate plus vaccines can temporarily interrupt lymphoid depletion and normal B cell development in bursa follicles, but this is usually followed by B cell repopulation and histological regeneration (Castro et al., 2009; Iván et al., 2001). Ezeokoli et al. (1990) reported that the IBD vaccine, of which they did not explain the type, caused serious lesions in the bursa in 3-7 days, but the bursa tissue was completely healed after 15 days, and there was no difference between the vaccinated group and the control group. In addition, another study reported that necrosis in the follicles partially disappeared, and the B lymphocyte population was recovered by 40-80% in 7 weeks (Kim et al., 1999). In the current study, it is observed that the bursa fabricius lesion score is mild to moderate in the first sampling after vaccination (Table 3, Figure 2). In the second samplings after vaccination, however, all groups revealed a decrease in bursal lesion scores (27-61 %). This was interpreted as an indication of lymphoid depletion decrease and histological regeneration in bursal follicles. With this improvement, which was observed at different levels in five different farms, it was thought that restoration of the immune response could be contributed as a result of the normalization of bursal functions, which provides a necessary micro-environment for the diversification of B cells with their immunoglobulin genes. Because the duration of immunosuppression and restoration of the humoral immune response are reported to be associated with the regeneration of BF after vaccination (Castro et al., 2009). In the study investigating the relationship between IBDV-induced bursal damage and the humoral immune response against Brucella abortus in SPF chickens, it was emphasized that the probability of being immunocompetent is low until more than half of the bursal repopulation rate is achieved (Edwards et al., 1982). In another study, it was reported that the alleviation of bursal lesions and the increase of B cell repopulation were faster in chickens inoculated with the vaccine strain than the virulent strain, and bursal damage caused by both applications resulted in a decrease in antibody synthesis (Kim et al., 1999). Based on this information, a positive correlation emerges

between the alleviation of bursal lesions and the humoral immune response. Therefore, in the present study, it was commented that regeneration in bursal histological architecture may have positive effects on the immune system.

CONCLUSION

In this study, it was concluded that the bursal lesion score resulting from the administration of the commercial intermediate plus vaccine originating from WF2512 did not rise to very high levels, and the lymphoid depletion decreased while the regenerative changes increased in the bursal follicles over time. It was also noted that the regression in vaccine-related bursal lesions in all farms was statistically significant when it was 30% or more. In the field, very virulent strains characterized by the continuous development of antigenicity and virulence of IBDV are seen as the cause of high mortality and economic losses due to long-term and severe suppression of the immune system. In order to prevent this, it may become inevitable to fight with less attenuated intermediate plus or hot vaccines by considering farms where IBD outbreaks occur as endemic. In such cases, the degree of bursal damage, the time it takes for lesions to heal, and the length of the rearing period should all be considered when choosing a vaccine.

ACKNOWLEDGMENT

Ethical approval:

The Ethics Committee of Experimental Animal Production and Research Center of the Faculty of Veterinary Medicine of Selcuk University (SÜVDAMEK) approved the study's ethical compliance (Approval No: 2021/ 144)

Conflict of interest: The authors have no conflict of interest.

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